

# ON THE DEVELOPMENT OF BIOPHYSICAL MODELS FOR SPACE RADIATION RISK ASSESSMENT

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## ABSTRACT

Experimental techniques in molecular biology are being applied to study biological risks from space radiation. The use of molecular assays presents a challenge to biophysical models which in the past have relied on descriptions of energy deposition and phenomenological treatments of repair. We describe a biochemical kinetics model of cell cycle control and DNA damage response proteins in order to model cellular responses to radiation exposures. Using models of cyclin-cdk, pRB, E2F's, p53, and G1 inhibitors we show that simulations of cell cycle populations and G1 arrest can be described by our biochemical approach. We consider radiation damaged DNA as a substrate for signal transduction processes and consider a dose and dose-rate reduction effectiveness factor (DDREF) for protein expression.

## INTRODUCTION

Theoretical models of initial damage to DNA have now reached a mature stage of development with Monte-Carlo track simulation codes that account for details of DNA structure, the hydration shell that interacts with DNA and most ionization and radical diffusion processes (Nikjoo *et al.*, 1997; and Holley and Chatterjee, 1996). For describing cellular endpoints such as cell death, chromosome aberrations, or cell growth and arrest, models of the initial induction of lesions and phenomenological descriptions of lesion repair have been used to correlate experimental data for dose responses and the time development of cellular effects. However, everyone knows that the deleterious outcomes that follow radiation exposure are mediated and modulated by gene expression. Mathematical modeling of gene expression is a well-known approach in molecular biology, however has received little attention in the study of radiation effects. Biochemical kinetics approach to modeling of mRNA and protein expression can be performed by computer simulation, including the solutions of non-linear differential equations that arise in a biochemical approach (Hargrove, 1995, Lauffenburger and Linderman, 1993). Important developments have been in studies of receptor binding and signaling (Lauffenburger and Linderman, 1993) and in studies of the behaviors of oscillatory enzymes (Goldbeter and Caplan, 1976). The role of diffusive processes in protein transport has also been considered (Goldbeter and Caplan, 1976; Lauffenburger and Linderman, 1993). The role of stochastic processes when low concentrations of molecular species are involved (Goss and Peccoud, 1998) may be important for signal transduction processes following low dose or low dose-rate exposures of cells. The mathematical description of covalent modification of enzymes (Goldbeter, 1981; Stadtman and Chock, 1977) predicted the importance of kinase activity in molecular controls related to cell cycle control and cancer including the large amplification of signals that may occur (Hunter, 1995). In this paper, we develop a mathematical description of several cellular processes using a biochemical model of the kinetics of cell cycle control and G1 checkpoint proteins.

## MATHEMATICAL MODEL OF CELL CYCLE CONTROL

The pathways that control the cell cycle (Strauss *et al.*, 1995) can mathematically be defined to successfully predict additional molecular interactions that modify the cell cycle. We have developed a model that incorporates sequential activation of cyclin-cdk complexes and their inhibitors. Identification of cyclin B and its associated cyclin dependent kinase cdk1 (cdc2) led to several mathematical descriptions of the molecular interactions that control the G2/M transition (Tyson, 1991; Novak and Tyson, 1993). These authors differed in their treatments of the number of intermediate kinetic steps related to dimer formation between cyclins and cdk's, the description of cyclin degradation, and control of cdk phosphorylation sites. Two models of G1 control have been discussed (Obeyesekere *et al.*, 1995; Hatzimanikatis *et al.*, 1995) which have considered the pRb protein, however they did not consider other cyclins and cdks or their inhibitors. We describe the relationship of cdk4, cyclin D, pRb, E2F, downstream kinases, and cdk inhibitors such as p16 and p21. We use the hypothesis that release of E2F/DP heterodimers from pRb provides a mechanism for the induction of downstream cyclins. We describe using non-linear differential equations, the periodic behavior and sequential activation of cyclin-cdks via coupled limit cycles (un-damped oscillatory solutions) and their transient behaviors. Our kinetic model uses compartments that describe the activation of each cyclin-cdk complex. Compartments are coupled by the release of the transcription factors E2F's from pRb sequester upon phosphorylation of pRb by an upstream cyclin-cdk complex. Expression of cyclin D correlates directly with non-mutated pRb (Sherr and Roberts, 1995) and we assume the cyclin D synthesis rate is proportional to the hypophosphorylated form of pRb in cells with normal pRb. Our model predicts that cyclin E is the primary regulator of cyclin A. Although this has not yet been observed directly, this conclusion is consistent with present experimental results. To complete the cell cycle and simulate its periodic behavior we require the dephosphorylation of pRb and the reformation of complexes of pRb with E2F's (Welch and Wang, 1995).

The model kinetic equations for cyclin synthesis and degradation, cyclin-cdk interactions, transcription factor control, and cdk inhibitor induction can be written in a compact form as given in Table 1. Here we are using an indexing subscript that links proteins of a similar family or of a similar regulatory nature. In Table 1,  $k_{ij}$  is rate constant for binding of molecules  $i$  and  $j$ ,  $v_s$  and  $v_D$  ( $v_D^P$ ) are synthesis and degradation rates, respectively. The  $k^P$  ( $r^P$ ) and  $k^Q$  ( $r^Q$ ) are rates of phosphorylation or de-phosphorylation, respectively. The  $k_F$  is rate of re-formation of a pRb/E2F complex denoted [pRb-E2F]. Concentrations of molecules with 'P' superscript are activated form and with superscript '0' inactive form. pRb is assumed to be dephosphorylated and to re-form dimers with E2F's in G2/M as controlled by cyclin B-cdk1 complex. The concentration of cyclin and cdk's are denoted  $[Cyc_i]$  and  $[cdk_j]$ , respectively with the index  $i$  corresponding to cyclins D, E, A, etc. and the index  $j$  corresponding to cdk4, cdk2, etc. Transcription factors are denoted  $[TF_i]$  and the transcription factors released by pRb are denoted  $[E2F_i]$ . pRb, E2F, and cdk levels are assumed to be constant throughout the cell cycle (Welch and Wang *et al.* 1995). We do not consider individual members of the pRb family in the present model. In Table 1 the equations that control the time rate of change of phosphorylated pRb, denoted  $[pRb^P]$ , and the free form of E2F are shown with the other forms of these molecules are solved by conservation. The formalism incorporates multiple inhibitors denoted as  $Ink_\alpha$  for cdk4 and cdk6 inhibitors such as p16 and p18 (Lukas *et al.*, 1995). Inhibitors bind specific cdk's or cyclin/cdk complexes. Synthesis and degradation rates reflect signals from extra-cellular milieu. We have made a numerical study on cell progression by finding limit cycle solutions to the model. Values of rate parameters leading to periodic oscillations of protein concentrations are found by local stability analysis of the steady-state solutions of the model equations. Since we consider the sequential activation of 4 cyclin-cdk complexes, cell phase arrest corresponding to low or high cyclin-cdk complex activity at several points in the cell cycle are possible. Limit cycles found for the cyclin D-cdk4 complex determine the limit cycles of downstream kinases which are dependent on the release of E2F's to switch from low activity modes.

Table 1. Molecular Kinetics Equations for Cell Cycle Progression and Regulation.

$$\begin{aligned}
 d[\text{Cyc}_i]/dt &= v_{Si} [\text{TF}_i] - v_{Di}^0 [\text{Cyc}_i] - \sum_j k_{ij} [\text{Cyc}_i][\text{cdk}_j] \\
 d[\text{Cyc}_i^P]/dt &= \sum_j v_{Dij} [M_{ij}^P] - v_{Di}^P [\text{Cyc}_i^P] \\
 d[\text{cdk}_j]/dt &= - \sum_i k_{ij} [\text{Cyc}_i][\text{cdk}_j] + \sum_i v_{Dij} [M_{ij}^P] + \sum_i v_{Di}^0 [M_{ij}^0] - \sum_\alpha k_{j\alpha} [\text{cdk}_j][\text{Ink}_\alpha] + \\
 &\quad \sum_\alpha v_{D\alpha} [\text{Ink}_\alpha - \text{cdk}_j] \\
 d[M_{ij}^0]/dt &= k_{ij} [\text{Cyc}_i][\text{cdk}_j] - (k_{ij}^P + v_{Di}^0) [M_{ij}^0] + k_{ij}^Q [M_{ij}^P] - \sum_\beta k_{ij\beta} [\text{KI}_\beta][M_{ij}^0] + \\
 &\quad \sum_\beta v_{D\beta} [\text{KI}_\beta - M_{ij}^0] \\
 d[M_{ij}^P]/dt &= k_{ij}^P [M_{ij}^0] - (v_{Dij} + k_{ij}^Q) [M_{ij}^P] \\
 d[\text{pRb}^P]/dt &= \sum_j r_{ij}^P [M_{ij}^P][\text{pRb} - \text{E2F}_{i+1}] / (K + [\text{pRb} - \text{E2F}_{i+1}]) - r_{G2/M}^Q [M_{G2/M}^P][\text{pRb}^P] \\
 d[\text{E2F}_{i+1}]/dt &= \sum_j r_{ij}^P [M_{ij}^P][\text{pRb} - \text{E2F}_{i+1}] / (K + [\text{pRb} - \text{E2F}_{i+1}]) - k_{F_{i+1}} [\text{E2F}_{i+1}][\text{pRb}^0] \\
 d[\text{Ink}_\alpha]/dt &= v_{S\alpha} [\text{TF}_\alpha] - v_{D\alpha} [\text{Ink}_\alpha] - \sum_j k_{j\alpha} [\text{cdk}_j][\text{Ink}_\alpha] \\
 d[\text{Ink}_\alpha - \text{cdk}_j]/dt &= k_{j\alpha} [\text{cdk}_j][\text{Ink}_\alpha] - v_{D\alpha} [\text{Ink}_\alpha - \text{cdk}_j]
 \end{aligned}$$

In Figure 1a, we show the percentage of protein expression as a function of time for cyclins E and A, and these results are compared with the data of Ohtusbo *et al.* (1995). Although they are not presented, the expression of free E2F's and cyclin kinases will follow closely the peaks in cyclins D, E, and A. Figure 1b shows the results of solutions for the expression of cyclin D, p16 and the hyperphosphorylated form of pRb. These calculated results are compared with the data of Tam *et al.* (1994) for expression of p16. The differences between the predictions of our model and experimental measurements are largest after the S phase peak where the cell cultures become de-synchronized. Cyclin D is differentially expressed, while cyclins E, A, and B show similar behaviors in individual cell types. In Figure 1c we show comparison of the model for cyclin E and cyclin A expression in HeLa cells (Pagano *et al.*, 1992) which do not express cyclin D. The good agreement of calculations with the above experiments demonstrates that our model can describe the sequential activation of 4 distinct cyclins by using the controlled release of transcription factors from tumor suppressor proteins such as members of the pRb family.

The growth kinetics of cell populations can be described by age-maturation diffusion equations mass-action rate equations, or discrete-time matrix methods. Here, if  $n_i(t)$  denotes the number of cells in phase  $i$ , and the duration of the phase denoted as  $\tau_i$ , the following set of differential equations describe the time-evolution of cell phase populations:

$$\begin{aligned}
 \frac{dn_{G1}}{dt} &= \frac{2}{\tau_M} n_M - \frac{1}{\tau_{G1}} n_{G1} & \frac{dn_S}{dt} &= \frac{1}{\tau_{G1}} n_{G1} - \frac{1}{\tau_S} n_S \\
 \frac{dn_{G2}}{dt} &= \frac{1}{\tau_S} n_S - \frac{1}{\tau_{G2}} n_{G2} & \frac{dn_M}{dt} &= \frac{1}{\tau_{G2}} n_{G2} - \frac{1}{\tau_M} n_M
 \end{aligned} \tag{1}$$

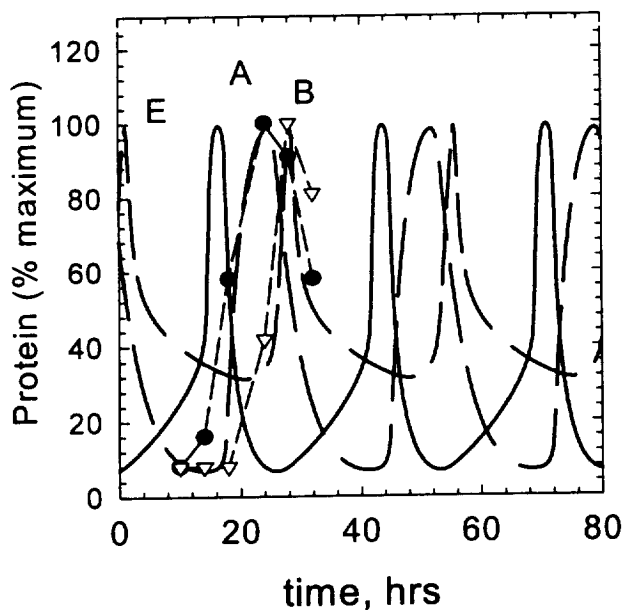


Figure 1a. Expression of cyclins E, A, and B in model and experiment for cyclin A and B (symbols).

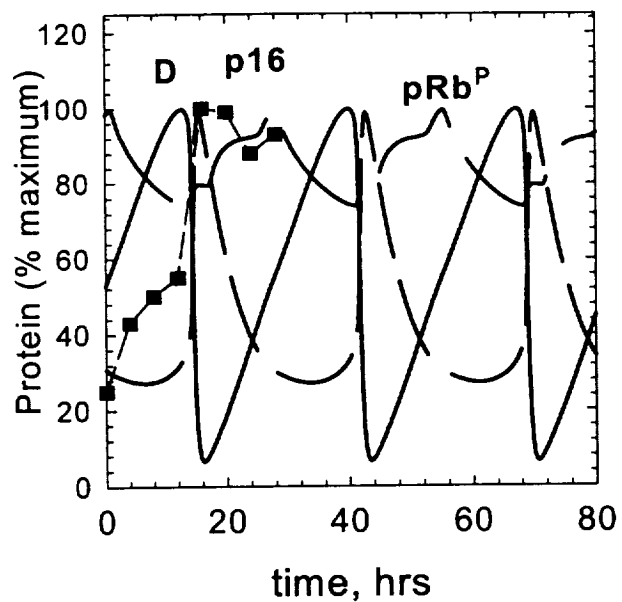


Figure 1b. Expression of cyclins D, p16, and pR in model and experiment for p16 (symbols).

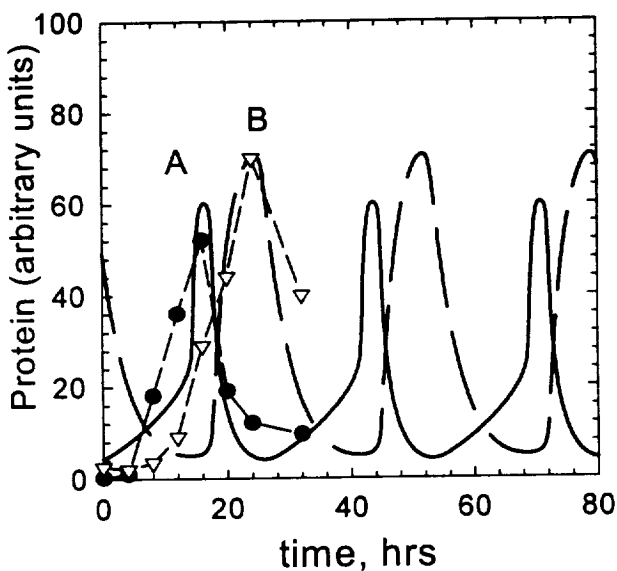


Figure 1c. Expression of cyclin E and A in model and in experiment (symbols).

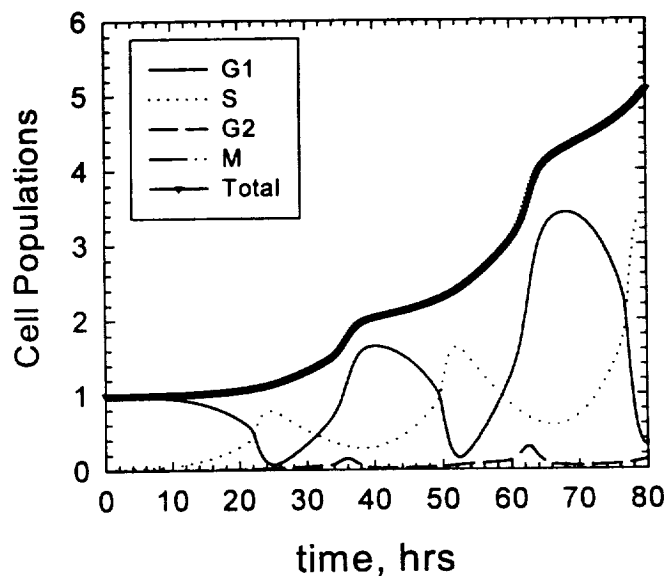


Figure 1d. Model prediction of cell phase kinetics for cells synchronized at M/G1 border.

where the factor of 2 for the G1 population in equation (1) accounts of doubling at mitosis. The mass-action equations with constant rate coefficients will reflect exponential growth, however no fluctuations in individual phase populations will occur after about 2 mitosis which is inconsistent with normal cell growth. In order to relate molecular controls on cell cycle progression to cell population kinetics, we replace the constant coefficients in equation (1) with coefficients that are proportional to the activation of a cyclin-cdk complex for the G1/S and G2/M transitions

$$1/\tau_s \propto M_{E/cdk2}^P ; \quad 1/\tau_{G2} \propto M_{B/cdk1}^P \quad (2)$$

with results shown in Figure 1d. This approach allows molecular switches described by our model to control progression of cell phase populations. This provides the mechanism for our model to directly relate protein fluctuations to cell phase population kinetics. Future applications will consider the loss of control of the mid-G1 restriction point and acceleration of G1 duration following oncogene activation or loss of tumor suppressor genes.

## P53 EXPRESSION AND SIGNAL TRANSDUCTION

Early events in acute tissue responses are driven by cell killing and delays in cell division in highly proliferating cells. Cell killing can be separated into necrosis and apoptosis. These are distinguished by observations of swollen cell size, inflammation, and shrunken chromatin for necrosis and DNA laddering, fragmented nuclei, and lack of inflammation for apoptosis. It is well known that in most cell types the protein p53 controls the apoptotic response to radiation. Apoptotic cell death is prominent in many tissues important for acute health effects including lymphocytes, intestinal crypt cells, and bone marrow or colony forming units. Because of the high selection for p53 functional inactivation in human cancers, many cell culture models are deficient in apoptotic cell death. Dose-rate effects in apoptosis has been studied in a few cases and indicate less sparring with low LET radiation than what is normally observed for cell killing. Also, there are noted differences in molecular interactions of p53 and other molecules in important signal transduction pathways between human p53 and mouse p53 including the raf and MAPK proteins (Steegenga, *et al.* 1996). The importance of such differences in radiation responses has not been investigated. Signal transduction following DNA damage by radiation is mediated by p53 coupling to the site of DNA breaks or DNA repair complex's (Reed *et al.*, 1995). Several outcomes are possible during signal transduction (Zhan *et al.*, 1994; Canman *et al.*, 1995) including growth arrest, programmed cell death (apoptosis) and also interference or cooperation with other signaling pathways as shown schematically in Fig. 2. It is believed that several of the p53 induced genes (PIGS) control the regulation of oxidative damage, ultimately leading to apoptosis (Polyak *et al.*, 1997). The up-regulation of latent p53 tetramers by post-transcriptional regulation is through the coupling of p53 tetramers to the DSB repair complex [ $C_{rep}$ ] and converts it to the active form (Hupp and Lane, 1995). The activated form functions as a transcription factor. The PIGS are then regulated by [ $p53^A_{tet}$ ].

We now discuss a mathematical model of the kinetics of p53 functions following DNA damage. Many of the details of these functions have not been established experimentally, and we make several simplifying assumptions in our model. First, because the tetramerization of p53 has been observed to be rapid relative to other cellular processes we consider only p53 tetramers. Second, following the ideas of Hupp and Lane (1994) we model the transactivation of p53 as an allosteric transition mediated by covalent modification of its carboxyl terminus and do not consider other phosphorylation sites in the present model. In many cell lineage's, the presence of ssDNA or a DNA repair complex, leads to the activation of the trans-activating form of p53. We assume the presence of a DSB repair complex is the signal for activation of p53 in our present model. The latent form of p53 is known to undergo rapid turnover with a half-life of about 25 minutes and the active form much longer-lived. The regulation of the transcription of p53 is modulated by the oncogene mdm2, which is transcribed following p53 activation. We write for the time-rate of change of the latent form of p53,

$$\frac{d[p53^L]}{dt} = k_{tet} - v_D^L[p53^L] - k_A[signal][p53^L] + k_L[p53^A] \quad (3)$$

and for the time-rate of change of the active form

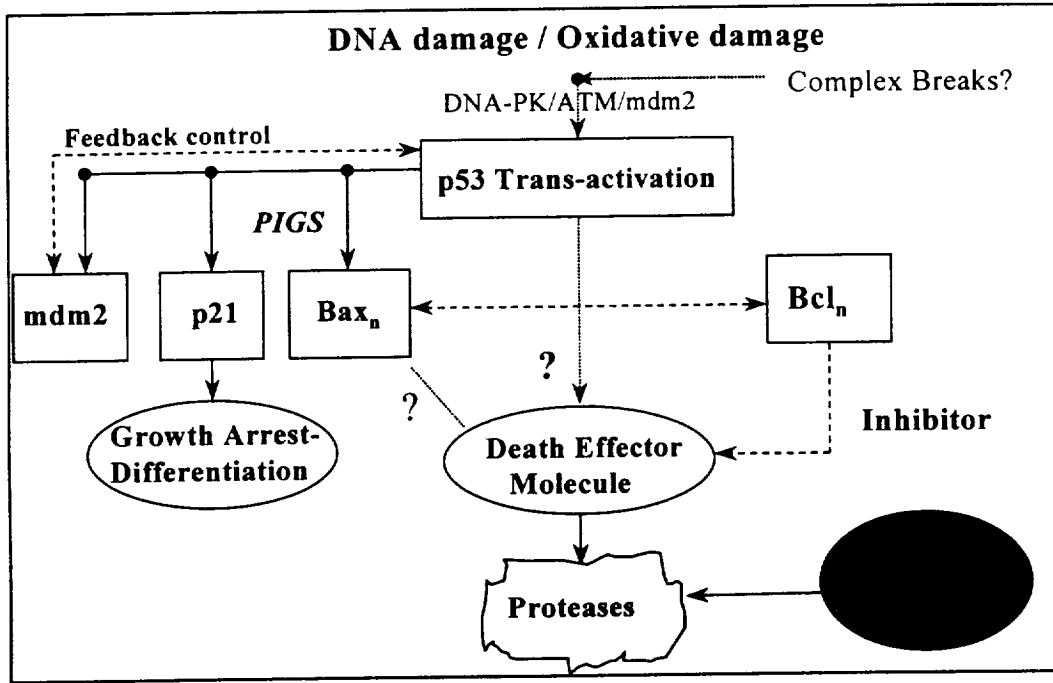


Fig. 2. Schematic of model reaction pathway of DNA damage leading to p53 expression, apoptosis or growth-arrest.

$$\frac{d[p53^A]}{dt} = k_A[signal][p53^L] - v_D^A[p53^A] - k_L[p53^L] - k_M[p53^A][mdm2] \quad (4)$$

where the  $v_D$  are degradation constants and the  $k$  are rate constants for (de)activation by conformation shifts of p53 or binding to mdm2 ( $k_M$ ). Similar equations can be written for the transcription of mdm2 and binding of the mdm2 protein to activated p53. The binding of a single mdm2 protein to a p53 tetramer is believed not to block completely the tetramers function. We thus consider active forms of p53 that are complexed with one or more mdm2. We assume that p53 continues to function as a transcription factor, although at a reduced efficiency, until all 4 sites are bound by mdm2. We ignore any cooperative effects (positive or negative). Rate constants for mdm2 binding are then unchanged as each binding site becomes filled. The p53 transcription of other genes is reduced at the geometric rate as these sites are filled. We bypass the intermediate step of the mdm2 RNA transcript that is easily included and should have only a small effect on modeling kinetics of the protein.

The induction of DNA DSB's is linear with dose for all dose-regimes of interest. Track structure studies indicate that the initial number of complex damages as defined by local damage clusters near the position of a DSB increase with LET or ionization density (Nikjoo *et al.*, 1997, Holley and Chatterjee, 1996). Repair pathways are assumed to be dependent of the damage type which accounts for the slower kinetics observed in DSB with high LET radiation. The repair of DSB's of type  $j$  is described by

$$\frac{d[DSB_j]}{dt} = \alpha \frac{dDose}{dt} - k_1[DSB_j][E_{rep}] \quad (5)$$

Where  $\alpha$  is the rate of formation of DSB's (about 40 DSB's per Gy of X-rays) and the time-rate of change of the repair-enzyme is described by

$$\frac{d[E_{rep}]}{dt} = -k_1[DSB][E_{rep}] + k_2[C_{rep}] \quad (6)$$

and of the repair-complex,

$$\frac{d[C_{rep}]}{dt} = k_1[DSB][E_{rep}] - k_2[C_{rep}] \quad (7)$$

with the initial condition  $E_0 = [E_{rep}] + [C_{rep}]$ . The reaction scheme described by equations (5)-(7) describes a dose dependent reaction velocity with saturation occurring at high doses (zero-order kinetics) and first-order kinetic at low doses. Studies of recombination repair of DSB's indicate that there are several reaction pathways available including single strand annealing (SSA), and homologous and non-homologous recombination (Roth *et al.*, 1985; Fishman-Lobel *et al.*, 1992). We are studying the effects of repair pathway competition in our mathematical model. We also are exploring the role of base-sequence near the site of DBS's which may effect processing such as SSA leading to deletions (Fishman-Lobel *et al.*, 1992). We couple the  $[C_{rep}]$  to p53 as the 'signal' in equations (3) and (4).

The kinase inhibitor p21 is normally found in quaternary complexes with the cyclins, cdk's, and proliferating cell nuclear antigen (PCNA) (Namba *et al.*, 1995; Li *et al.*, 1994). Increases in p21 concentration cause G1 arrest (Dulic *et al.*, 1994) through inhibiting G1 cyclin associated kinase activity. In our model we examine p53 dependent p21 upregulation after DNA damage as described by

$$\frac{d[mRNA]}{dt} = r_T + r_{p53}[p53^A] - r_D[mRNA] \quad (8)$$

where  $r_T$  and  $r_D$  are the basal rates of transcription and degradation, respectively, and  $r_{p53}$  is the rate constant for coupling of the transcription factor p53 to the p21 promoter. The time rate of change of p21 is given by

$$\frac{d[p21]}{dt} = v_S[mRNA] - v_D[p21] - k_I[p21][M^0_D] - k_I[p21][M^0_E] \quad (9)$$

where  $v_S$  and  $v_D$  are the synthesis and degradation rates of p21, respectively. The last two terms in Eq. (9) are the coupling of p21 to the G1 cyclin-cdk complexes. For the background levels of p21 we use its known half-life of 30 minutes and assume that there is normally about one molecule of p21 in each of the G1 cyclin complexes. Fits of the model to the data of Bae *et al.* (1995) for the time course of p21 and p53 expression in exposures of normal lymphoblast cells are shown in Figure 3. The comparison of the model to experiments at high doses determines model rate constants and allows for an alternate assessment of low dose-rate effects. In Figure 4 we show predictions of the model for the induction of DSB's, p53 and p21 expression at low dose-rate. These results indicate that although much sparring is seen in DNA damage repair, the persistence of p53 expression allows for a build-up of PIGS that would lead to apoptosis, growth arrest, and ultimately to harmful tissue effects. Modifications of the present model will be required to describe the modulation of DNA damage signaling proteins for time courses greater than 10 hrs after DNA damage, including the role of phosphorylation or other conformation changes of p53 throughout the cell cycle that are known to affect its DNA binding and transcription activity.

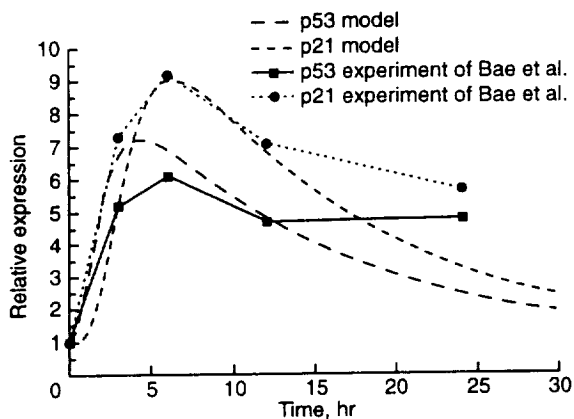


Figure 3. Model calculations of p53 and p21 expression versus time after 6.3 Gy acute exposure as fit to data of Bae *et al.*, 1995.

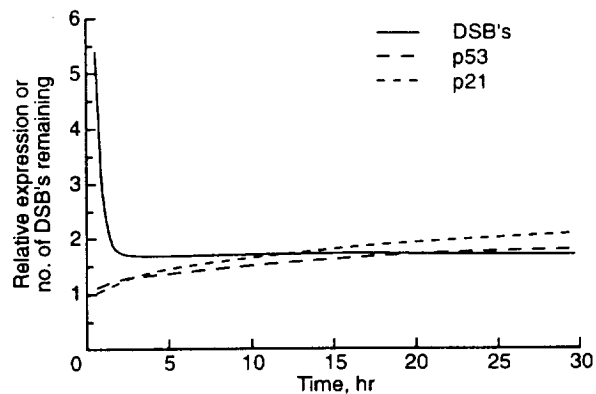


Figure 4. Predictions of model for number of DSB's remaining and relative expression of p53 and p21 for 0.1 Gy/hr gamma irradiation.

Genomic instability after radiation exposure would likely modify p53 levels because of the observed high sensitivity of p53 to DNA damage (Nelson and Kastan, 1994). These low dose-rate results predict that there is no threshold for p53 induction in agreement with observations of Lane (1998) for low dose-rate acute exposures. In Figures 5 we show comparisons of the model for acute exposures of 6.3 Gy in early G1 and at G1 + 6hrs. Figure 5a display a large G1 arrest in agreement with experiment (Dulic *et al.*, 1994). The results of the model are in general agreement with the Western blot analysis of Dulic *et al.* (1994). The results of Figure 5b show only minimal G1 arrest for the same exposure level. Here, the non-linear expression of kinase activity prevents the inhibitor in causing arrest. These results suggest that for asynchronous populations, G1 arrest is dependent on timing of signaling pathways and non-linear accumulation of cyclin associated kinases.

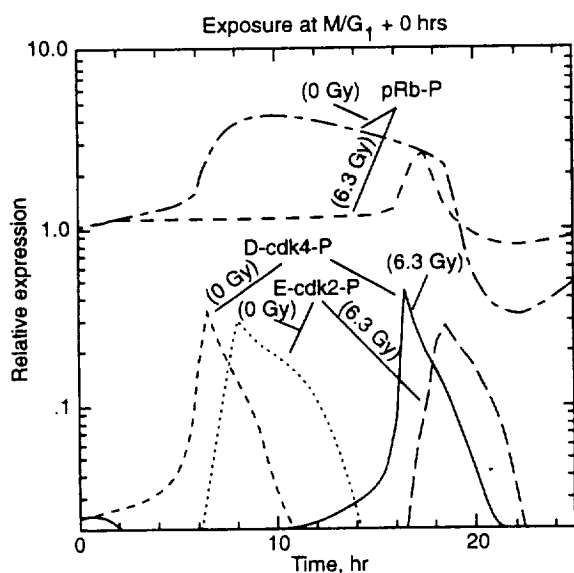


Figure 5a. Calculations of relative expression of several proteins after acute exposure of 6.3 Gy at the M/G1 border.

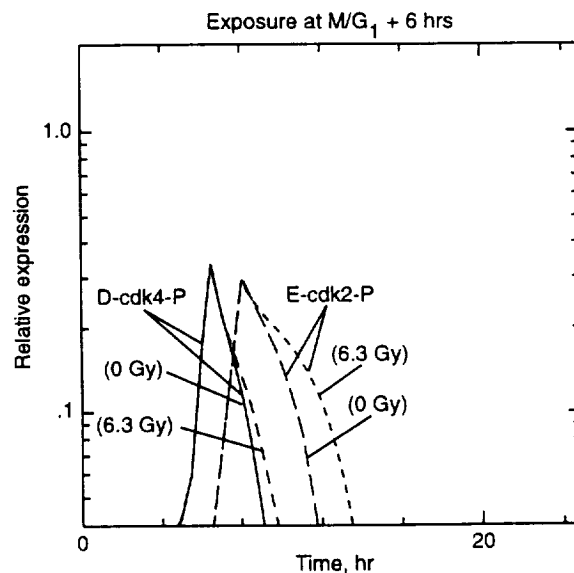


Figure 5b. Same as Fig. 5a for exposure at 6 hours past M/G1 border.



## CONCLUSIONS

Modification of intracellular signaling patterns and their effect on cell cycle control is a potential determinant in radiation response and must be adequately understood for extrapolating risk models to low dose-rates and the complicated radiation fields in space. We have described a mathematical model of cell cycle control through regulation of the related proteins and the coupling of this model to DNA damage through the p53 signal transduction pathway. Knowledge of protein-protein interactions, most importantly the residues involved in kinase activity and the possibly large number of binding proteins, may place limitations on models. However, biochemical models serve as a useful approach to consider these interactions and to model dose and dose-rate dependent responses. In future work, the model discussed here will be extended to describe exposures to asynchronous cell populations. The role of p53 regulation by DNA damage many hours after exposure, and the description of the G2 arrest will be determinants in this description. A similar mathematical description can be applied to study other early events in the apoptosis pathway including p53 induction of the apoptosis inhibitor molecules, BAX and BAX formation of hetero-dimers with members of the apoptosis promoting Bcl family.

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